

Replication of Sialodacryoadenitis Virus of Rat in LBC Cell Culture

Brief Report

By

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With 5 Figures

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Summary

Sialodacryoadenitis virus of rat readily propagated and induced marked cytopathic effect in a rat cell line, LBC cell culture, which provides a sensitive, practical assay system for viral infectivity and neutralizing antibody, and a satisfactory source of the virus.

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Coronaviruses (8) infect a wide variety of animal species including avian and human beings and cause respiratory disease, hepatitis, enteritis or encephalitis. From rats, rat coronavirus (RCV) (7) and sialodacryoadenitis virus (SDAV) (1) have been isolated. By intranasal inoculation RCV causes a fatal pneumonitis in newborn rats and SDAV induces sialodacryoadenitis in adult rats. They have been shown to share common antigen(s) with mouse hepatitis virus (MHV). High incidences of antibody to MHV in rat colonies (2, 5) suggest that RCV and SDAV might be perpetuating in rat colonies without any apparent illness, while outbreaks of sialodacryoadenitis are common in laboratory rat colonies (4, 6, 9), causing serious problems in breeding and experimentation.

Information on the virology of RCV and SDAV are still limited because these viruses can be propagated only on primary culture of rat kidney cells but not on any established cell lines (1, 7). Recently, we reported a successful propagation of RCV in a rat cell line LBC with marked cytopathic

effect (CPE) (3). The present brief report deals with replication of SDAV, strain 681 (1), on the LBC cell culture.

SDAV, strain 681 (1), was kindly supplied in 1976 by Dr. A. M. Jonas, Comparative Medicine, Yale University, New Haven, U.S.A. The LBC cells, established from a spontaneous mammary tumor developed in a Lewis rat (3), were grown at 37° C in Eagle's minimum essential medium (MEM) containing 10 per cent fetal calf serum (FCS) and kanamycin (0.06 mg/ml). The FCS concentration was reduced to 5 per cent for maintaining the cells and harvesting the virus. The cell monolayers prepared in 50-ml culture bottles were washed once with phosphate buffered saline (PBS) and inoculated with 0.2 ml of virus material. After virus adsorption at 37° C for 60 minutes, the inoculated cultures were fed 5 ml of maintenance medium and incubated at 37° C.

Cytopathic change was first detected on infected cultures at 48 hours postinoculation (p.i.), and 72 hours p.i. syncytia appeared as reported on infected primary culture of rat kidney cells (1). Passages of SDAV in LBC cell monolayers were readily carried out at intervals of 3 days with undiluted culture fluid. After 6 passages much more remarkable CPE developed within 24 hours p.i. CPE induced by SDAV in LBC cells consists of syncytia and their detachment from the glass surface (Figs. 1A and 1B). The infectivity of infected culture fluid was estimated by inoculation into LBC cells prepared in 13 × 100 mm test tubes, showing $10^{7.0}$ 50 per cent tissue culture infective doses (TCID₅₀)/0.2 ml at the 10th passage level.

The LBC cell monolayers grown in coverslips were inoculated with the virus passaged 10 times on LBC cells, fixed at 24 hours p.i. with cold acetone. The fixed samples were treated with anti-SDAV rat serum (1:10) supplied by A. M. Jonas at 37° C for 60 minutes, and then overlaid with fluorescein isothiocyanate-conjugated anti-rat IgG rabbit serum (Miles Biochemicals, U.S.A.) (1:10) at 37° C for 60 minutes. Strong fluorescence was demonstrated within cytoplasm, especially in perinuclear area of syncytia (Fig. 2), as reported on replication of RCV in LBC cells (3).

Serial 2-fold dilutions of the anti-SDAV rat serum in MEM were mixed with an equal volume of the virus material (200 TCID₅₀/0.2 ml) and the mixtures were incubated at room temperature for 60 minutes. Then, 0.2 ml of the mixtures were inoculated into LBC cells prepared in test tubes. The LBC passaged-virus at the 10th level was found to be neutralized by the antiserum giving a titer of 1:320.

The supernatant of infected culture fluid was examined by electron microscopy (Hitachi H-600A) after negatively stained with 2 per cent uranyl acetate. As shown in Fig. 3, numerous virions, 120 to 160 nm in diameter, with characteristic spikes were demonstrated.

Fig. 4 illustrates representative viral growth curves which were obtained in LBC cell cultures infected with the virus at an input multiplicity of

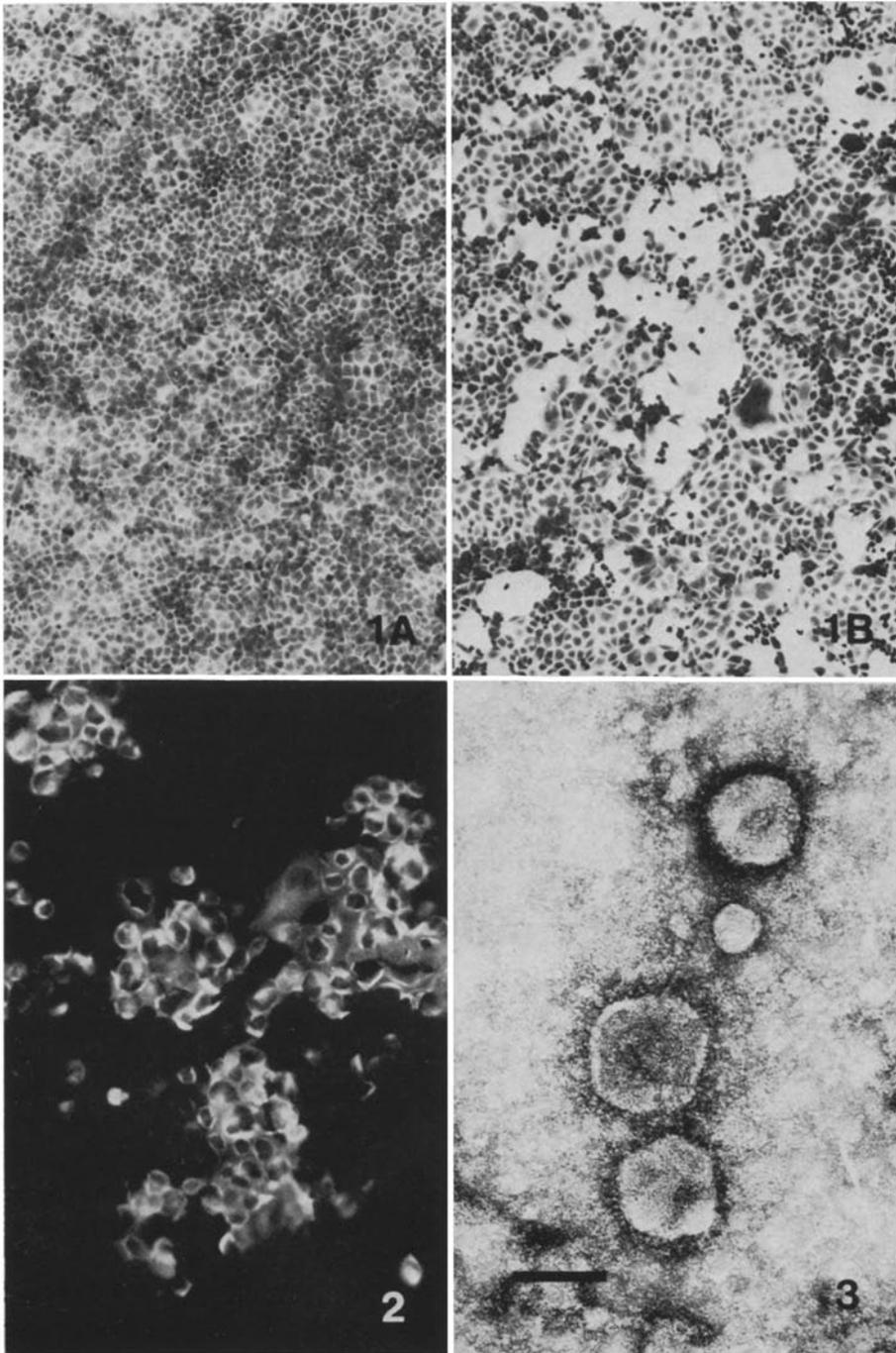


Fig. 1. Cytopathic effect of sialodacryoadenitis virus in LBC cell culture (Hematoxylin and eosin stained). Cells uninfected (*A*), and 24 hours after infection with the virus (*B*)

Fig. 2. Immunofluorescence of LBC cells 24 hours after inoculation with sialodacryoadenitis virus

Fig. 3. Negatively stained virus particles from the infected culture fluid of LBC cells ($\times 125,000$). Bar indicates 100 nm

10 TCID₅₀ per cell. CPE was first observed at 12 hours p.i. and became complete in the inoculated cultures at 24 hours p.i. The titer of cell-associated virus, after a gradual decline during the first 4.5 hours of incubation, showed a rise within 6 hours p.i. followed by a rapid exponential increase up to

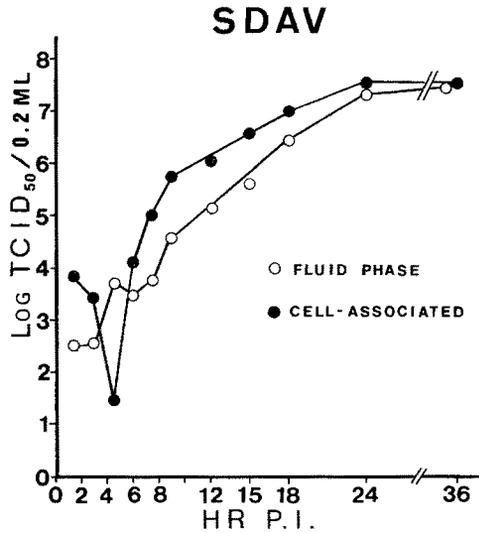


Fig. 4. Growth curves of SDAV strain 681 in the LBC cell culture. Cell cultures prepared in 50-ml bottles (1.0×10^6 cells/bottle) were infected with 10 TCID₅₀/cell of the virus, washed three times with PBS after virus adsorption at 37° C for 1.5 hours, fed 5 ml of maintenance medium and incubated at 37° C. At intervals, infectivity was determined with culture fluid and cells pooled from two bottles. For the assay, the cells were washed with PBS, and frozen and thawed in 5 ml of fresh medium. Those materials were centrifuged at $800 \times g$ for 15 minutes before infectivity assay

9 hours p.i. and small rise to plateau of $10^{7.5}$ TCID₅₀/0.2 ml at 24 hours p.i. Increase of extracellular virus titer was slower than that of cell-associated virus and reached a plateau of $10^{7.5}$ TCID₅₀/0.2 ml at 24 hours p.i. The growth curve experiment showed that a release of newly formed virus from the cells was dependant upon the progress of CPE. CPE in LBC cells appeared earlier and was clearer than that in primary culture of rat kidney cells (4). The infectivity titers obtained in LBC cells were higher than those reported previously on primary culture of rat kidney cells (1, 4).

The LBC cells were shown to be more sensitive to SDAV strain 681 and to yield high titered virus, and would be a useful tool for studying on coronaviruses from rats including RCV and SDAV.

Acknowledgements

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References

1. BHATT, P. N., PERCEY, D. H., JONAS, A. M.: Characterization of the virus of sialodacryoadenitis of rats: a member of the coronavirus group. *J. Inf. Dis.* **126**, 123—130 (1972).
2. FUJIWARA, K., TANISHIMA, Y., TANAKA, M.: Seromonitoring of laboratory mouse and rat colonies for common murine pathogen. *Exp. Anim.* **28**, 297—306 (1979).
3. HIRANO, N., ONO, K., INOUE, A., MURAKAMI, T., TAKAMARU, H.: Replication of rat coronavirus in a rat cell line, LBC. *Arch. Virol.* **75**, 301—304 (1985).
4. KOJIMA, A., FUJINAMI, F., DOI, K., YASOSHIMA, A., OKANIWA, A.: Isolation and properties of sialodacryoadenitis virus of rat. *Exp. Anim.* **29**, 409—418 (1980).
5. NAKAGAWA, M., SAITO, M., SUZUKI, E., NAKAYAMA, K., MATSUBARA, J., NUTO, T.: The years-long survey on pathogen status of mouse and rat breeding colonies. *Exp. Anim.* **33**, 115—120 (1984).
6. NUNOYA, T., ITABASHI, M., KUDAOW, S., HAYASHI, K., TAJIMA, M.: An epizootic outbreak of sialodacryoadenitis of rats. *Jpn. J. Vet. Res.* **39**, 445—450 (1977).
7. PARKER, J. C., CROSS, S. S., ROWE, W. P.: Rat coronavirus (RCV): a prevalent, naturally occurring pneumotropic virus of rats. *Arch. ges. Virusforsch.* **31**, 293—302 (1970).
8. SIDDELL, S. G., ANDERSON, R., CAVANAGH, D., FUJIWARA, K., KLENK, H. D., MACNAUGHTON, M. R., PENZAERT, M., STOHLMAN, S. A., STURMAN, L., VAN DER ZEIJST, B. A. M.: Coronaviridae. *Intervirology* **20**, 181—189 (1983).
9. UTSUMI, K., ISHIKAWA, T., MAEDA, T., SHIMIZU, S., TATSUMI, H., FUJIWARA, K.: *Lab. Anim.* **14**, 303—307 (1980).
10. YAMAGUCHI, R., TAGUCHI, F., YAMADA, A., UTSUMI, K., FUJIWARA, K.: Pathogenicity of sialodacryoadenitis virus for rats after brain passages in suckling mice. *Jpn. J. Exp. Med.* **52**, 45—48 (1982).

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